GROWTH AND DEVELOPMENT OF MYCORRHIZAE OF SUGAR MAPLE (ACER SACCHARUM MARSH.)

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Abstract

Beaded rootlet growth in *Acer saccharum* is thought to be caused by intermittent growth due to alternately favorable and unfavorable moisture conditions during the season rather than by mycorrhizal infection. Both beaded and nonbeaded rootlets are mycorrhizal-infected, and rootlets growing in the deeper soil levels where moisture conditions do not fluctuate rapidly are nonbeaded. Differences found in the activity of rootlets growing in hummocks and depressions appeared to be related to soil moisture conditions found there. This paper describes (*a*) the anatomy of metacutinized root tips during dry soil conditions, and the relationship of this condition to the development of constrictions between beads; (*b*) the extrastelar anatomical characteristics of beaded and nonbeaded rootlets; (*c*) the morphology of the mycorrhizal fungus in its relationship to rootlet anatomy; (*d*) the extramatrical mycelium of the mycorrhizal fungus found in the soil of the rooting zone (the extramatrical mycelium is thought to arise from fungal hyphae and vesicles produced in live rootlets which are released to the soil from disintegrating rootlet cortical tissue); and (*e*) an hypothesis regarding the life history of this type of vesicular–arbuscular mycorrhizal fungus.

Introduction

Beaded rootlet growth as shown in Fig. 1 is characteristic of the genus *Acer*. The criterion for such growth used in this paper was the presence of constrictions at intervals on the rootlets. Most investigators have concluded or implied that the beaded condition was caused by the presence of a fungus within the cortical cells. Hacskaylo (5, 6) speculated that beading was caused by periods of arrested growth of the roots followed by periods of active growth as the result of changes in environmental conditions. Beaded endotrophic mycorrhizae of the vesicular–arbuscular type have been reported for *Acer rubrum* L. (1, 2, 9, 14), *A. negundo* L. (2, 15), *A. saccharinum* L. (14, 15), *A. platanoides* L. (8), and *A. saccharum* Marsh. (12, 15).

This paper describes the anatomy and some aspects of growth of the endotrophic mycorrhizae of sugar maple (*A. saccharum* Marsh.). The terms "rootlet" and "mycorrhiza" are used here interchangeably and correspond to Lyford and Wilson's (13) use of "mycorrhiza" to designate the highest order of nonwoody red maple roots.

Materials and Methods

In 1963, mycorrhizal collections were made in an old-growth stand of northern hardwoods on the Upper Peninsula Experimental Forest near Marquette, Mich. The forest type corresponds to No. 27, sugar maple, as delineated by the Society of American Foresters (20), in which sugar maple is the dominant tree in the stand both in reproduction and established trees.

Mycorrhizae were collected from A_0, A_2, and B_2 layers of the soil (a well-drained sandy loam classified as Munising sandy loam), placed in plastic bags, and taken to the laboratory where they were carefully washed. They

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were then floated in water and examined under the stereoscopic microscope. Individual mycorrhizae were sketched to show their general appearance, excised with a scalpel, and transferred to Randolph's fixative (11) for 24–48 hours. They were fixed under vacuum to allow better penetration of the fixative. The tertiary butyl alcohol schedule of Johansen (11) was used to dehydrate the rootlets before they were embedded in tissuemat at 60 °C. A hypodermic syringe was used to transfer solutions in small vials so that the small rootlets would not be lost during the dehydrating and infiltrating processes. Mycorrhizae were cut 12 microns thick in serial sections. By Johansen's quadruple staining schedule, which was used to differentiate the fungus from the content of the host cells, the various elements stained as follows.

Element	Color
Hyphae within the cortical cells	Green
Hyphal connections outside rootlets	Violet
Nucleoli	Red
Nucleus	Light green
Xylem cell walls	Pink
Inclusion bodies in the endodermal and hypodermal cells	Yellow
Phloem	Light green
Cytoplasm of meristematic cells	Bright green
Root cap cells	Reddish brown
Cortical cell cytoplasm	Pale yellow
Sporangioles	Brownish yellow-green

Photomicrographs were made with a Linhof Technika camera, Panatomic-X film, a Bausch and Lomb Trinocular microscope, and Spencer Ortho illuminator.

Results

Growth, External Morphology, Internal Anatomy, and Metacutinization of Mycorrhizae

Although growth of the mycorrhizae of sugar maple reaches a maximum in the spring and early summer (16), much variation was noted while I was examining individual sites (Table I). Much of this variation was related to variable microtopography and consequent soil moisture distribution.

TABLE I

Percentage of white (indicating recent or current growth) growing tips found at different sites during growing season of 1963* in hummocks and adjacent depressions

Site	June 25		July 8 or July 19		August 5	
	Hummock	Depression	Hummock	Depression	Hummock	Depression
Moderately well-drained moist site	60	64	72	35	37	54
Well-drained site	41	43	86	48	59	72
Excessively well-drained site, droughty	43	44	26	74	22	24

*Based on observations of 80 or more root tips per time and site.

PLATE I

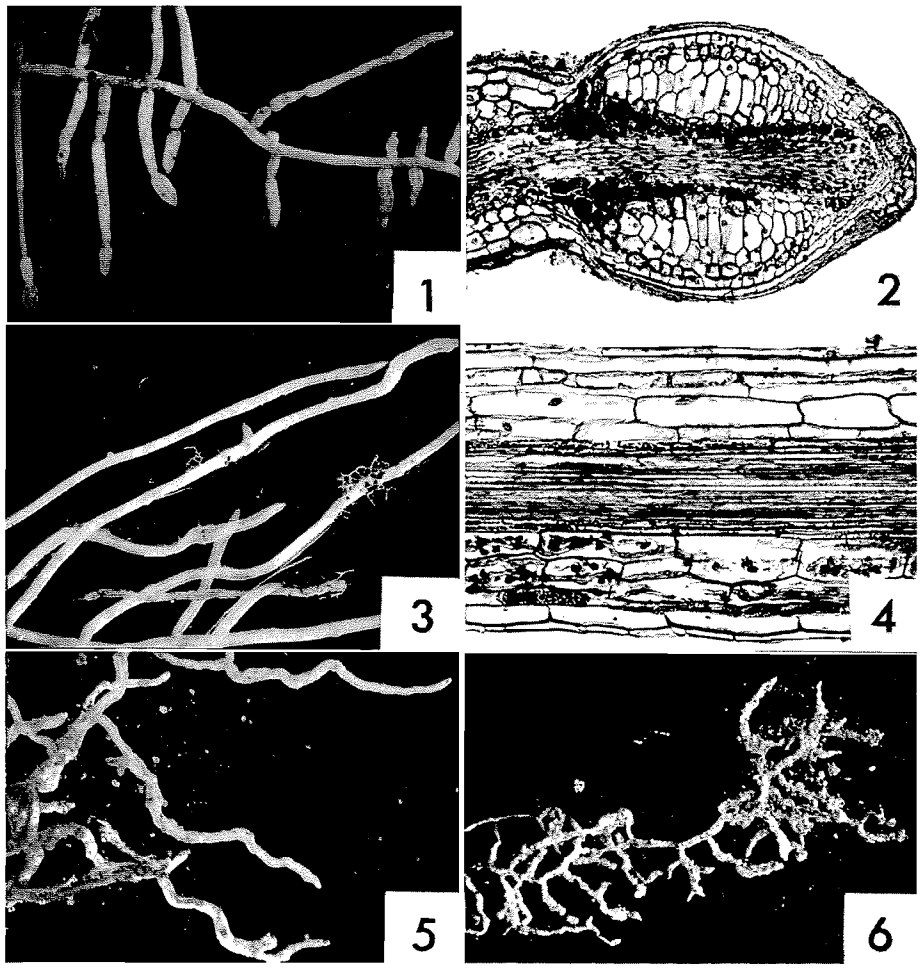
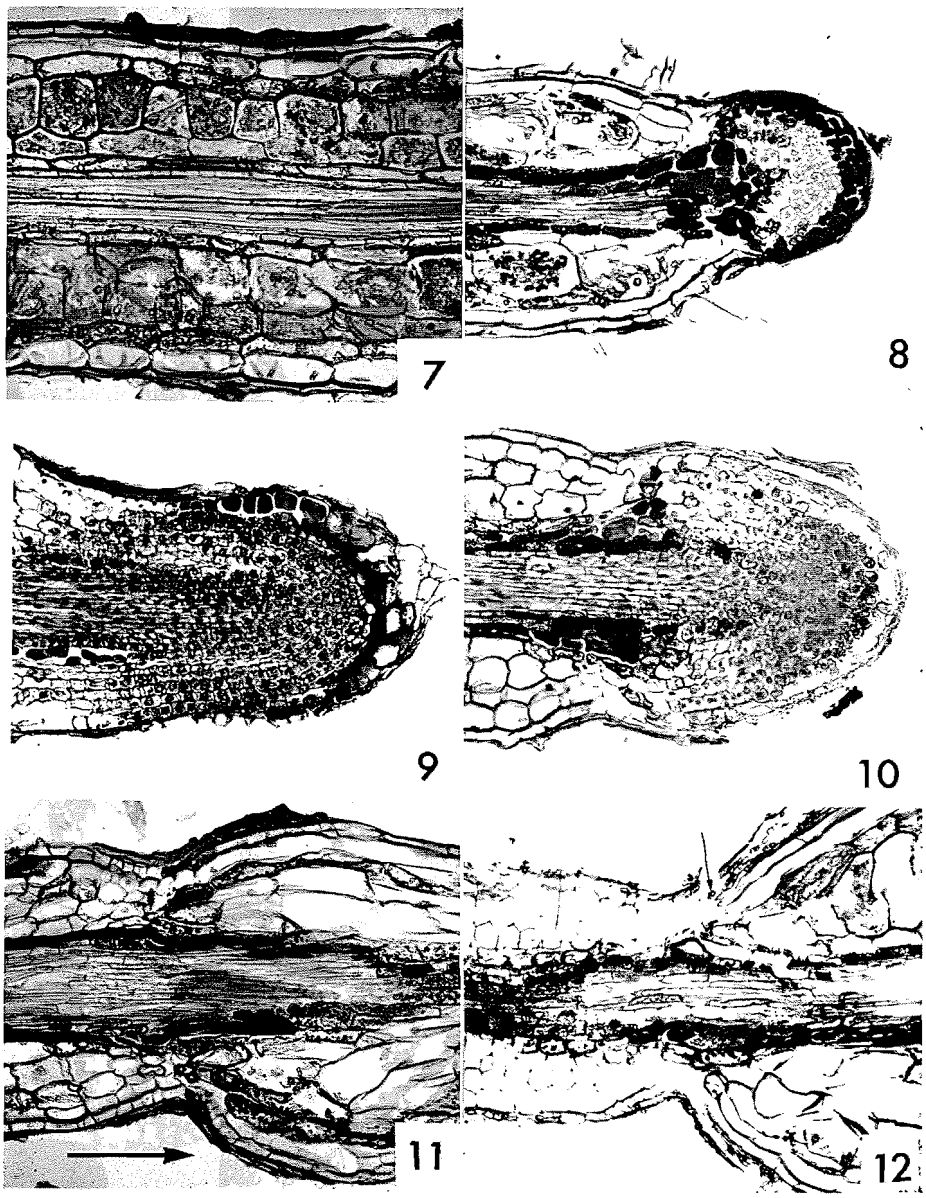


FIG. 1. External view of beaded rootlets. ×1
FIG. 2. Longitudinal section of dormant beaded rootlet. ×250
FIG. 3. External view of long white rootlet. ×1
FIG. 4. Longitudinal section of portion of long white rootlet. Note mycorrhizal infection of cortical cells in lower part of rootlet. ×250
FIG. 5. External view of rootlets from intermediate soil depth (12 in.). ×1
FIG. 6. External views of rootlets from lower level of rootlet occupancy in soil (24 in.). ×1



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The time required for sugar maple rootlets to become dormant or to proceed from dormancy to active growth can be very short. Morrow (16) described a situation in September when the soil moisture was below the wilting point and no rootlet activity could be detected. Three days of precipitation followed and 2 days later when rootlets were again examined, there was a striking resumption of growth which matched the growth peak found in the spring. In the present study, similar reflushing of rootlet (mycorrhizal) growth was observed several times during the summer of 1963 when enough rain fell to remoisten the humus layer for at least 3 days. As the humus became drier again, growth of mycorrhizae slowed and cutinization of the tips began. These cycles of alternate wetting and drying, with corresponding periods of mycorrhizal growth and dormancy, appear to be responsible for the beaded character of sugar maple mycorrhizae. When moisture conditions were favorable for continuous growth, mycorrhizae grew 30 mm or more in length without developing a constriction (Fig. 3).

Mycorrhizae growing in the A_2 and upper B_2 soil layers where moisture conditions did not fluctuate as rapidly as in the humus layers were not extensively beaded (Fig. 5), nor were root tips in this zone as heavily suberized.

In the lower B_2 horizon, mycorrhizal growth was distorted. Beaded growth was not present at this depth, and growth appeared relatively continuous (Fig. 6) since no constriction areas were present that would indicate breaks in growth.

Rootlets at all depths contained a mycorrhizal fungus in the cortical cells. A few rootlets had growing upon them a fine network of dark hyphal threads which differed from the extramatrical fungus described later. When sectioned, epidermal and hypodermal cells of older rootlets were commonly seen to contain thin hyphae very different in appearance from mycorrhizal hyphae found in cortical cells.

The nature of mycorrhizal growth on roots that occurred in hummocks differed considerably from that of roots in depressions (Table I). On one droughty ridge sampled in mid-July, 26% of the tips examined from a hummock were white, indicating current or recent growth, whereas in an adjacent depression, 74% of the tips were white. In a lowland hardwood area nearby, 72% of the root tips from a hummock were white; and in an adjacent depression where the water table was about 2 ft from the surface, only 35% had white tips. In both areas, soil moisture was higher in the depressions; but

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FIG. 7. Longitudinal section of rootlet intermediate in appearance between beaded type (Fig. 1) and long white type (Fig. 3). Compare shape of cortical cells in this rootlet with the elongate ones in the long white rootlet of Fig. 4. Cortical cells contain the mycorrhizal fungus while hypodermal cells in lower part of rootlet contain unknown fungus. ×250

FIG. 8. Longitudinal section of rootlet resuming growth. Note impregnated, deeply stained cells behind meristematic region. Large cortical cells contain mycorrhizal fungus. ×250

FIG. 9. Longitudinal section of growing rootlet. Compare with rootlets in Figs. 2, 8, and 10. ×250

FIG. 10. Longitudinal section of rootlet resuming growth after a dormant period. ×250

FIG. 11. Longitudinal section of the nodal area between beads. Note the outer suberized layer which is thinner toward the older bead and thicker toward the new one. The arrow indicates direction of growth. ×250

FIG. 12. Nodal area with break and fungal hyphae colonizing it. Longitudinal section. Arrow points to fungus hypha. ×250

in the lowland area the soil moisture content was so high that root growth was apparently inhibited. As this area became drier later in the season, additional root growth ensued in the depression and by early August the percentage of white tips rose to 54.

Suberized mycorrhizae were highly tolerant of drought. But when moisture conditions again became favorable, suberization of the root tips prevented immediate resumption of elongation, since the new growth had to rupture the suberized cell layers. The rupturing process appears somewhat similar to the development of lateral roots from the pericycle.

When the root tips became suberized and dormant, differentiation and development of the cortical cells occurred immediately behind the meristematic tissue (compare Figs. 8 and 9). Fully differentiated cortical cells were always separated from the meristematic region in the dormant mycorrhizae by a layer of impregnated cells (Fig. 8). This layer often appeared as an inverted cone and extended from the stele to the outer cutinized layer. In some mycorrhizae examined, the separation layer had not been extensively developed, but some impregnated cells were always present. When conditions again became favorable, the meristem resumed growth, and the impregnated cells remained in place and were later observed as the impregnated cells at the constrictions between beads. These cells may possess mechanical or chemical properties harmful to the mycorrhizal fungus present in the cortical cells since the fungus was never observed to penetrate this layer, either between beads or into the meristematic tissue at the apex.

After the new apex ruptured the suberized tip, a break in the epidermal layer, usually covered by the suberized outer layer, was observed (Fig. 10). The suberized layer covered the outer surface between two beads and was thinner toward the older bead and thicker toward the new one (Fig. 11). Sometimes the suberized layer was loose or poorly developed and ruptured between the beads (Fig. 12). These ruptures could possibly act as invasion routes for fungi since fungal hyphae were sometimes seen there (Fig. 12). The identity of the fungi is not known, but their small hyphal size indicated that they probably were not the same as the mycorrhizal fungus inhabiting cortical cells.

Rootlet Anatomy of the Nonstelar Tissues

The primary difference between the narrow roots of the long mycorrhizae (Figs. 3 and 4) and the short, swollen beaded growth (Figs. 1 and 2) was in the

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FIG. 13. Hyphal coiling in cortical cells. Tangential section. ×400

FIG. 14. Constriction of hypha passing through cell wall between two cortical cells. Tangential section. ×500

FIG. 15. Hyphae passing through cortical cell walls without constriction. Tangential section. ×500

FIG. 16. Hyphae with protuberances in cortical cells. Tangential section. ×300

FIG. 17. Section cut through hyphae which were coiled closely around nucleus. Longitudinal section. ×300

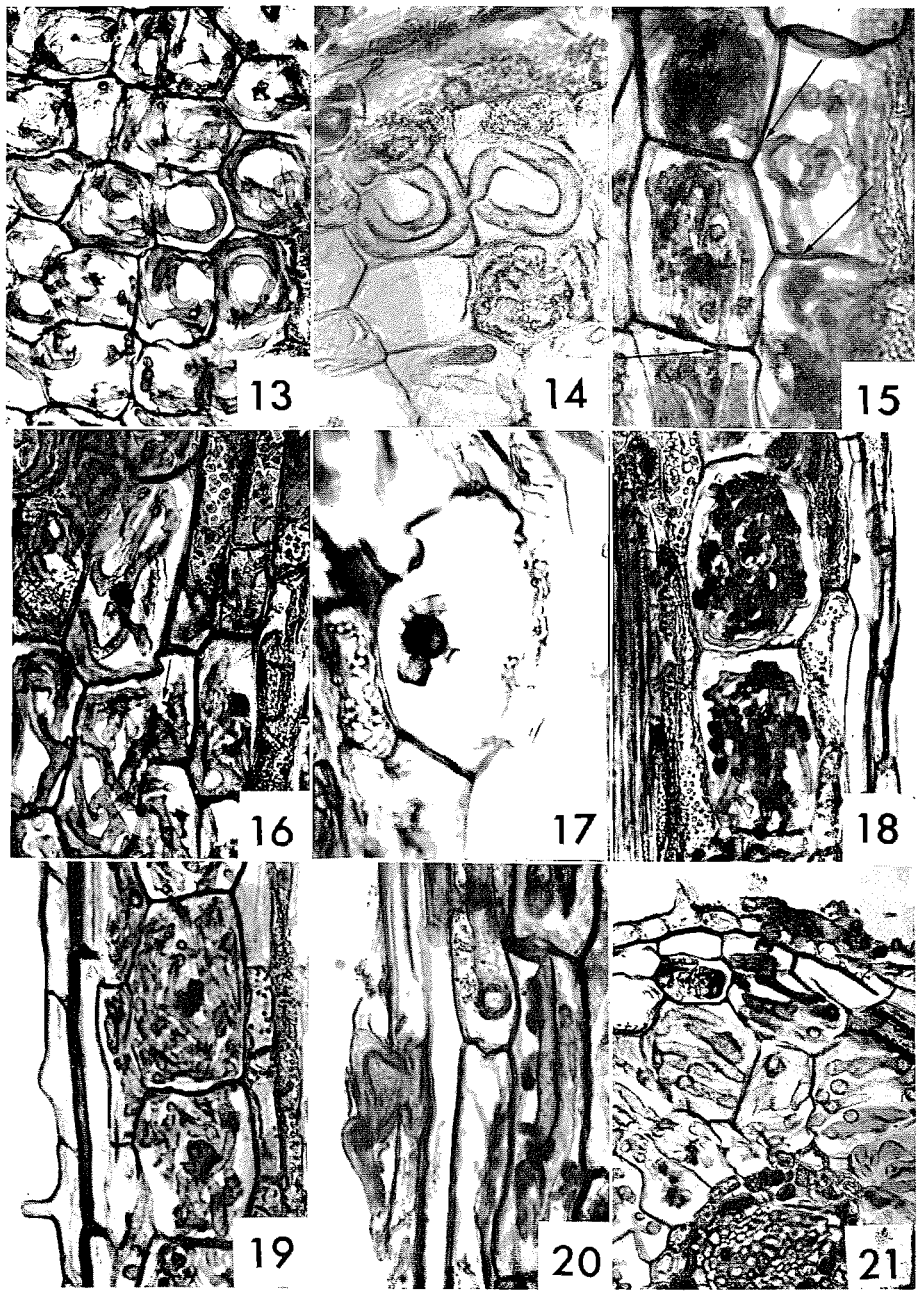
FIG. 18. Sporangioles in cortical cells. Longitudinal section. ×400

FIG. 19. Arbuscules in cortical cells. Longitudinal section. ×400

FIG. 20. Longitudinal section through appressorium at penetration point. ×500

FIG. 21. Hyphae in cortical cells below penetration point. Dark penetration hyphae stained violet while lighter hyphae in lower cells are stained green. Transverse section. ×400

PLATE III



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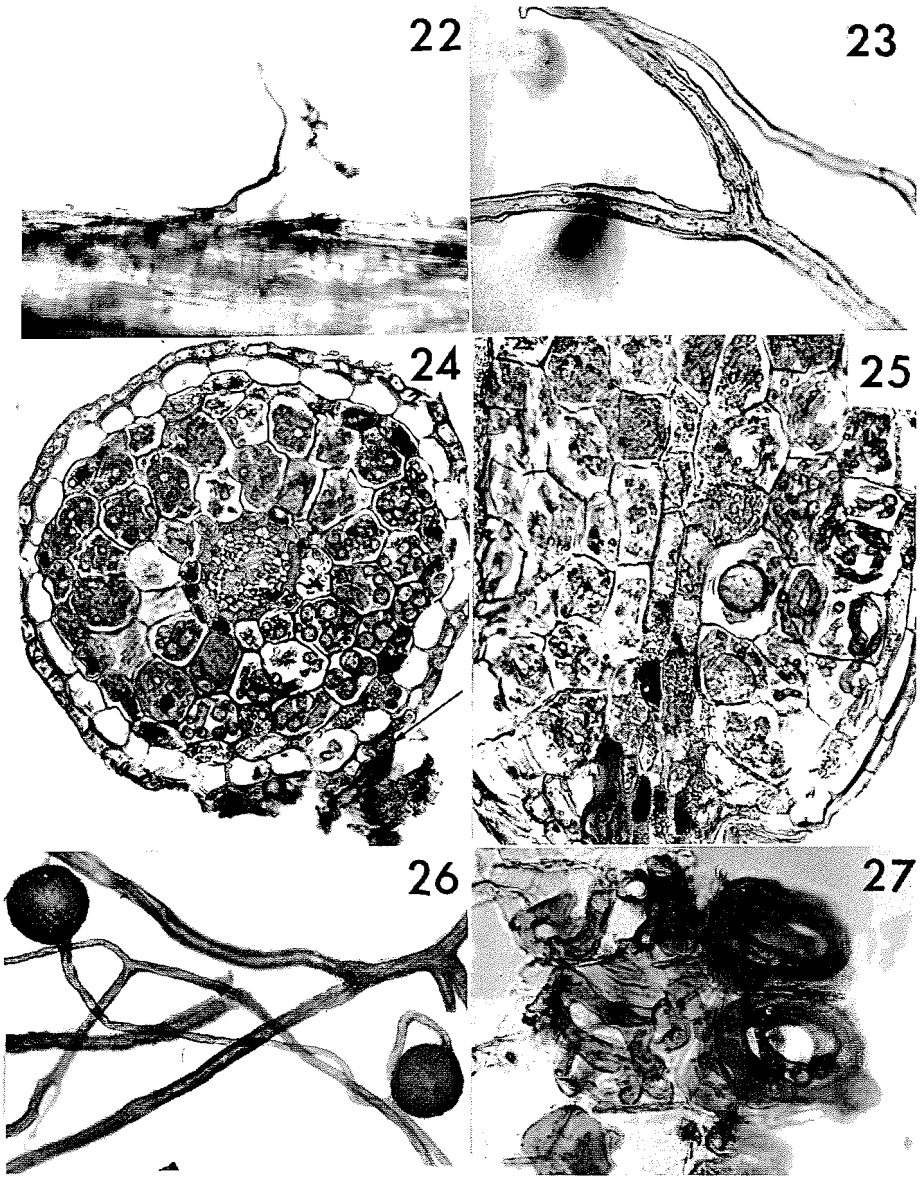

FIG. 22. Attachment of extramatrical mycelium to rootlet. Two unattached appressoria are out of focus in background. ×250

FIG. 23. Extramatrical mycelium. Double wall development. ×500

FIG. 24. Large hyphae and one vesicle in cells surrounding ingression point (arrow). Other fungal stages are located farther away. Transverse section. ×250

FIG. 25. Dark-staining hyphae and vesicles near ingression point, arbuscular-sporangiolar stages on other side of rootlet, longitudinal section. ×250

FIG. 26. Extramatrical mycelium, hyphae, and vesicles. ×250

FIG. 27. Hyphae in moribund rootlet. Longitudinal section, on freezing microtome. ×500

shape and numbers of cortical cells. In long mycorrhizae, cortical cells were elongate longitudinally whereas in the beaded mycorrhizae, the radial axis of the cortical cells became larger so that the cells were more cubelike. Mycorrhizae with intermediate morphological characteristics had cortical cells intermediate in shape between those of the long and beaded mycorrhizae (Fig. 7).

The relationship of the cell layers in long and beaded mycorrhizal types is the same (compare Figs. 2 and 4) and is as follows from surface to center (Fig. 28).

1. A single layer of epidermal cells.
2. A hypodermal layer with thickened walls on the outer longitudinal and radial walls.
3. A single layer of cortical cells containing inclusion bodies.
4. A layer of cortical cells one to four cells thick, the layer where the most abundant development of the mycorrhizal fungus occurs.
5. An endodermal layer containing inclusion bodies which stain similarly to those in the outer cortical layer.
6. The pericycle where new lateral growing points arise.
7. The vascular tissues.

Rootlet Anatomy and Fungus Morphology

Uninfected rootlets were rarely found in soil layers containing humus. Usually hyphae (Fig. 13), sporangioles (Fig. 18), and arbuscules (Fig. 19) could be found in single infected rootlets. Vesicles were abundant in some rootlets and rare in others.

Hyphae were always found in tissues adjacent to penetration points where the fungus was or had been connected to other hyphae outside the rootlet (Figs. 24 and 25). Hyphae leading away from the penetration point stained violet with the quadruple stain. As the hyphae proceeded further from the penetration point, their staining color changed to green. The difference in staining reaction can be seen in Fig. 21. The violet stain was concentrated in the fungal cell walls, whereas the green was more diffuse and stained both cell walls and cytoplasm. Hyphae penetrated directly through the epidermal and hypodermal layers and then ramified in the outer cortex cells. Hyphae were often observed as coils surrounding cortical cell nuclei (Fig. 17). Orientation of the coils usually paralleled the longitudinal axis of the rootlets, so that coiling was more readily seen in longitudinal sections (compare Fig. 2 with Fig. 21). Hyphae often possessed angular protuberances (Fig. 16).

Appressoria at the penetration points are irregular in shape, with one to many protuberances on the surface of each (Figs. 20 and 22). Appressoria stained violet. Arbuscules were seen in all stages of development in cortical cells. Sporangioles were attached to the arbuscules at angular protuberances (Fig. 18). This attachment was difficult to see because of the clumping of the sporangiolar material.

The hyphae progressed intracellularly. Sometimes there was a constriction in the hyphae that passed through cell walls but often there was none (Figs. 14 and 15). There was no indication that the cell walls were mechanically ruptured, and penetration holes between cells had the appearance of cell wall dissolution. Within a rootlet the hyphae never appeared to lead to an egress point. Multiple penetration points into single rootlets were common and

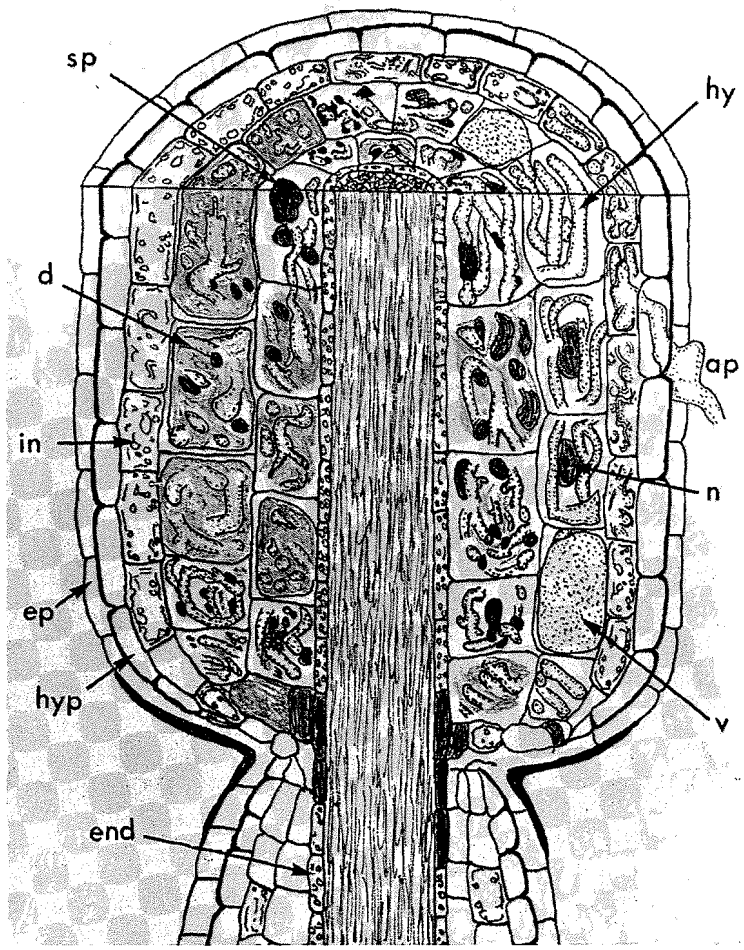

FIG. 28. Relationship of fungal forms to rootlet structure. *Ap*, appressorium; *d*, cortical cell containing hyphae believed to be in process of dissolution; *ep*, epidermis; *hy*, hypha; *hyp*, hypodermis; *in*, inclusion body; *n*, nucleus; *sp*, sporangiole; *v*, vesicle.

caused overlapping of developmental stages of the fungus, which made sections difficult to interpret.

The endophyte of sugar maple is of the intracellular Paris type (3). The cortex had an outer hyphal layer and an inner arbuscular layer. The hyphal layer was centered around the penetration point and was usually confined to the first two cortical layers beneath the hypodermis (Figs. 28 and 29). Invasion hyphae linked the hyphal layer through hypodermal and epidermal cells to the exterior at the penetration point. The hyphal layer extended longitudinally as much as 300 μ or more in both directions from the penetration point. In the second cortical layer the hyphae extended farther longitudinally than laterally. In the first cortical layer, inclusion bodies obscured hyphae and prevented accurate evaluation of their spread.

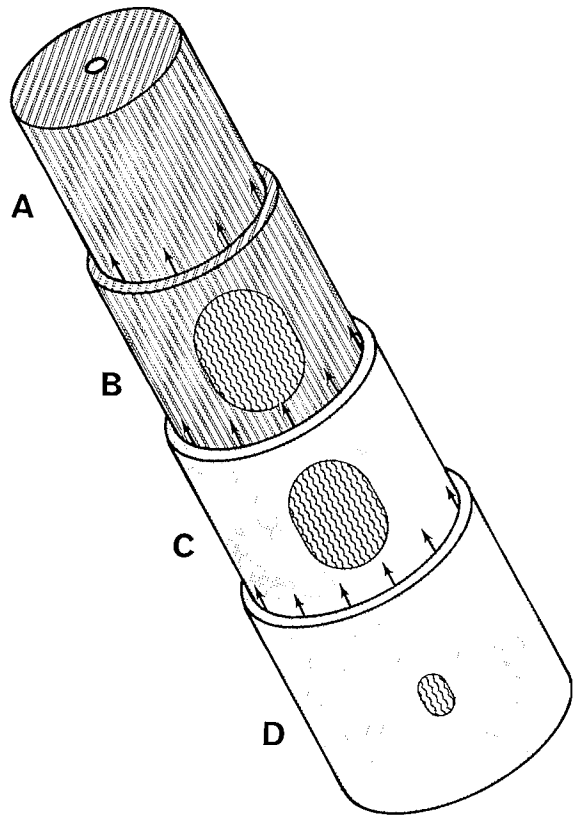

FIG. 29. Spatial arrangement of hyphal and arbuscule layers in rootlet. The four layers have been telescoped apart to show the overlapping of forms. Wavy lines indicate the hyphal layer, straight lines the arbuscule layer, and stippling the noninfected tissue. A, inner cortex and stele; B, mid-cortex; C, single layer of cortical cells containing inclusions; D, epidermal and hypodermal layers.

The arbuscular layer was not confined solely to the inner cortex but was also found at the longitudinal extremities of the hyphal layer. Arbuscular forms were never seen in cells containing inclusions in the outer cortical layer.

Vesicles were usually at the extremities of the hyphal system away from the penetration point but were sometimes observed in the inner cortical cylinder, occupying entire cells. Vesicles were occasionally seen in cells containing arbuscular–sporangiolar material.

Frozen microtome sections of dying rootlets revealed hyphae and vesicles in the hyphal layer surrounding the penetration point. Hyphae and vesicles in this layer stained intensely with trypan blue, and cytoplasmic contents were visible in many; but fungal structures in cortical cells of the arbuscular layer were obscure and disintegrating.

The Extramatrical Mycelium of the Mycorrhizal Fungus

A ramified hyphal system was often found in the humus layer associated with the rootlets, and connections were sometimes found between it and the

mycorrhizal hyphae within the rootlets (Fig. 22). These attachments appeared to be ephemeral, and often only the appressorium and a short piece of attached hypha were seen. Some attachments may have been broken during the rootlet washing process but, if so, they were consistently broken close to the rootlet surfaces. The extramatrical hyphal system was often very extensive and, visually, was one of the dominant members of the humus microflora.

These connections between extra- and intra-matrical mycelium on the surface of the rootlet were similar to the appressoria of some pathogenic fungi. The connections consisted of an irregular-shaped "foot" from which one or more penetration hyphae arose and entered the epidermis. Often, the appressoria had peglike structures which did not enter the epidermis. These may have been abortive penetration hyphae. Many rootlets had few or no root hairs, while others had many. Root hairs were rarely observed to act as infection courts.

The hyphae in the soil were of varying sizes. A main system of large thick-walled hyphae with branches of smaller size was a constant characteristic (Fig. 26). Often the large hyphae could clearly be seen to have double walls (Fig. 23). Angular protuberances on the thick-walled hyphae were seen. Nicolson (18) thought that these are remains from the disintegration of the small hyphae and that the large thick-walled hyphae are more permanent in the soil.

The large thick-walled hyphae ranged from 4.8 to 29 μ in diameter, and their thin-walled hyphal subdivisions ranged from 2.4 to 7.2 μ in diameter. The large hyphae were usually nonseptate and stained heavily with aniline blue or trypan blue in lactophenol. The small hyphae stained lightly and often had septa. Nicolson (18) and Mosse (17) described similar dimorphic hyphal systems for mycorrhizae of grasses and apple, respectively. On the ends of some of the thick-walled hyphae of intermediate size, nearly spherical to oval vesicles were observed (Fig. 26). These ranged from 26 to 122 μ in diameter. They were rough walled and had dense cytoplasmic contents which stained intensely with aniline blue.

Comparison of Extramatrical Mycelium and Intramatrical Mycelium

Because of the similarity between the extra- and intra-matrical mycelia (both systems have vesicles, varying hyphal sizes, variation in hyphal wall thickness, and angular protuberances on the hyphae), a comparison of them was made (Table II). Length of the largest hyphae that could be continuously traced without a break was similar in both systems. Maximum length of hyphae of the extramatrical mycelium was 9729 μ and the average of 12 systems measured was 5060 μ. Maximum length of hyphae of the intramatrical mycelium was 6790, and the average length of 11 systems was 4610 μ. Maximum widths were generally greater for extra- than for intra-matrical hyphae. The difference in hyphal width was due to the thicker wall of the extramatrical hyphae; their hyphal lumen width averaged slightly larger than those of the hyphae of the extramatrical mycelium. The number of vesicles on the extramatrical mycelium ranged from 0 to 6 and averaged 2.1, while the number on the intramatrical mycelium ranged from 0 to 17 and averaged 3.4. Extramatrical vesicles were usually larger; they averaged 73.7 μ in diameter compared with 41.3 μ for intramatrical vesicles.

TABLE II

Measurements (μ) of hyphae and vesicles of the intra- and extra-matrical mycelium

Rootlet number	Maximum length of large hyphae	Maximum hyphal width	Maximum hyphal wall thickness	Width of hyphal lumen	Number of attached vesicles	Maximum width of largest vesicle
			Intramatrical			
1	5550	8.4	0.5	7.4	0	—
2	6790	9.6	1.2	7.2	9	25.6
3	5420	18.0	1.2	15.6	3	40.8
4	3750	15.6	1.2	13.2	0	—
5	5720	16.8	.5	15.8	2	44.4
6	4000	12.2	1.2	9.8	0	—
7	3600	14.4	1.2	12.0	1	28.6
8	6000	12.2	1.2	9.8	17	55.2
9	3020	10.8	1.2	8.4	1	37.2
10	3140	10.8	1.2	8.4	4	57.6
11	3720	13.2	1.2	10.8	0	—
Average	4610	12.9	1.1	10.8	3.4	41.3
			Extramatrical			
1	9729	25.6	5.2	15.2	3	63.6
2	3672	21.6	6.0	9.6	2	86.4
3	5508	24.0	7.2	9.6	6	96.0
4	5008	18.0	3.6	10.8	0	—
5	3840	15.6	3.6	8.4	1	66.0
6	5780	15.6	2.4	10.8	5	60.0
7	3540	9.6	2.4	4.8	0	—
8	5570	22.6	4.8	13.0	0	—
9	1890	12.0	2.4	7.2	2	89.7
10	3330	12.2	2.4	8.4	2	64.0
11	9420	12.0	2.4	7.2	4	63.6
12	3430	15.6	2.4	10.8	0	—
Average	5060	17.0	3.7	9.6	2.1	73.7

Discussion

The observations presented here concerning mycorrhizal growth at different soil depths and in hummocky soils have led to a concept of the absorbing root system as a dynamic, ever-growing, ever-adjusting system constantly exploiting the soil moisture available to it. When the upper layer of the soil becomes too dry to support mycorrhizal growth, the tips metacutinize and become dormant. In the lower soil levels where moisture is still adequate, mycorrhizae continue to grow. When the upper soil becomes remoistened, the rootlets resume growth until diminishing moisture again slows or halts it. When excess moisture is present at the lower soil level, the mycorrhizae closest to the surface are the more active.

As the soil becomes drier at the lower levels, mycorrhizal activity may occur only at the lowest level of the root system. During drought periods, mycorrhizal development would be greater than normal at a greater depth in the soil profile. During an extended wet period, development of the root system would be at its maximum close to the surface where oxygen was plentiful. When an extended dry period is followed by an excessively wet period, rootlet injury might be expected in the lower soil profile since the root system has become overbalanced there. Although excess moisture, unless protracted, might not kill mycorrhizae, it could have the same physiological effect on

the tree as does drought through disruption of absorption and translocation functions.

The first partial cut in northern hardwood stands on moderately wet sites sometimes results in crown deterioration or dieback (4). These stands, formerly able to maintain a balance with soil moisture through transpiration, become disrupted in two ways (7). Soil close to the surface becomes drier through increased evaporation; and soil at the lower levels becomes wetter through decreased transpiration from the reduced overstory. These unfavorable influences may result in a drastic reduction in the area available for mycorrhizal development. If the reduction is severe enough, compensating reductions (dieback) in crowns may result.

Several authors (2, 8, 14) have either stated or implied that the beaded rootlets of maples were due to the presence of a mycorrhizal fungus. In this investigation, long nonbeaded rootlets as well as the beaded rootlets were found to harbor the fungus. The beaded condition was most apparent closest to the surface where moisture fluctuations were greatest. Only by growing rootlets aseptically can proof be obtained about whether a mycorrhizal fungus is necessary for bead formation. But the observations in this study indicate that very likely the mycorrhizal condition is not necessary and that beading is brought about by intermittent rootlet growth induced by soil moisture fluctuations, as Hacskaylo (5, 6) has conjectured.

Differential staining of penetration hyphae at ingression points has proved useful in locating these hyphae easily. The difference in staining reaction between the penetration hyphae (violet) and other hyphae within cortical cells (green) indicates that a basic difference in composition may exist in the cell wall of the hyphae. Johansen (11), in describing the quadruple stain, stated that lignified, suberized, or cutinized cell walls stained reddish while cellulose cell walls and the middle lamellae stained different shades of green.

Comparison of extra- and intra-matrical mycelium revealed several similarities. Perhaps the most surprising was the finding that lengths of primary large hyphae were similar in both systems. At first examination, extramatrical mycelium appears to be an extensive system, but when examined closely under the microscope it is seen to be a complex of smaller extramatrical mycelia. Each of such mycelial units has (1) a primary thick-walled hypha with secondary thick-walled subdivisions, and (2) thin-walled subdivisions some of which may originate from the primary and some from the secondary thick-walled hyphae. Vesicles may be present or absent. The extramatrical mycelial units have a tendency to coil; when they come into contact with other hyphae they become easily entangled and may appear to be an extensive, continuously connected system.

When the largest extramatrical hyphae were compared with the largest intramatrical hyphae, the dissimilarity in size was found to be due to the thicker cell wall of the extramatrical hyphae. The greatest difference between the extra- and intra-matrical mycelium was the larger vesicles of the extramatrical mycelium, a difference that could not be accounted for by cell wall thickness. When dying rootlets were examined, the only endophytic structures that resisted disintegration were the hyphae and vesicles in the area around the penetration point (Fig. 27). These often contained cytoplasmic contents.

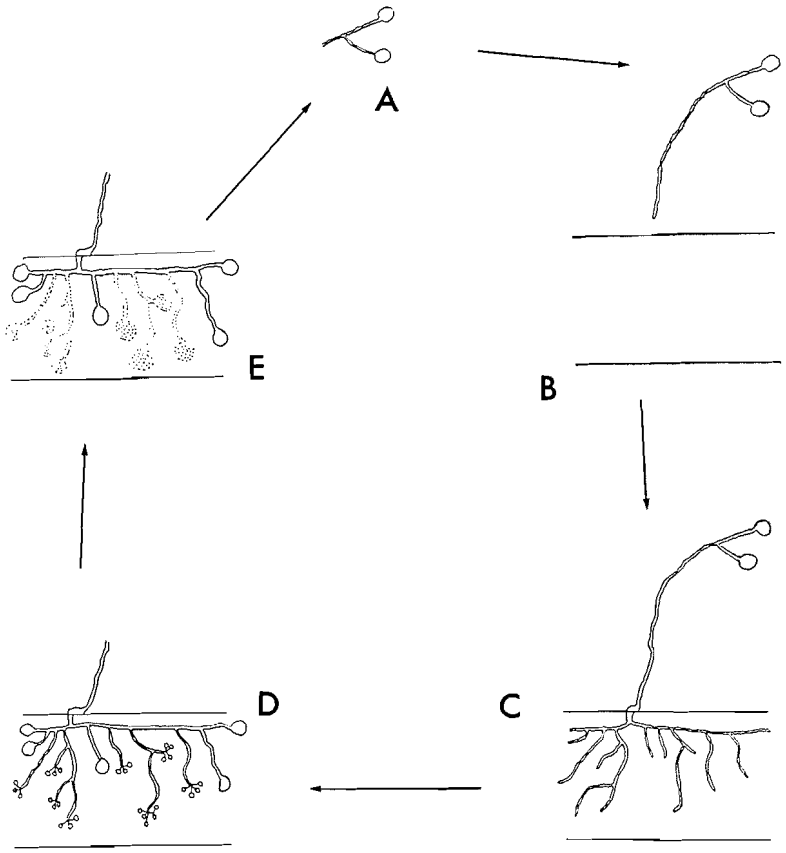

FIG. 30. Diagram illustrating possible life cycle of vesicular-arbuscular mycorrhiza of sugar maple. A, extramatrical mycelium in soil; B, extramatrical mycelium germinates near rootlet; C, hyphal penetration and spread within rootlet; D, development of vesicles and arbuscules on hyphal system (connection with extramatrical mycelium has usually been broken by the time this stage is reached); E, arbuscule stage disintegrates, and vesicles and some hyphae remain and are subsequently released into soil upon rootlet death or sloughing of tissue.

If hyphae and attached vesicles commonly survive rootlet death or the disintegration of cortex, an hypothesis can be constructed which differs from other hypotheses in that the fungus is not thought to play a role in the absorption of nutrients from the soil but instead acts to conserve nutrients already present in rootlets. Janse (10) and Schrader (19) have developed preliminary hypotheses that follow this concept. The author has developed their line of reasoning further into the following working hypothesis (see Fig. 30). The hyphae of the extramatrical mycelium in the soil around the rootlet penetrate the epidermal and hypodermal cells radially. Then the fungus multiplies within the cortex, developing an outer hyphal layer and an inner arbuscular layer. During the multiplication phase, connection is maintained with the extramatrical mycelium and materials are translocated from the extra- to the

intra-matrical mycelium. After the latter is established, the connection with the extramatrical mycelium is broken. The outer layer, however, maintains hyphal connections with the arbuscular layer. Nutrients are translocated via these hyphal connections from the arbuscular absorbing organs to vesicles that form at the extremities of the layer. After vesicle formation, connections between the hyphal and arbuscular layers are disrupted, and the arbuscular organs disintegrate and are absorbed by the cortical cells. Hyphae and vesicles in the outer cortex become dormant and subsequently are either released into the soil upon rootlet death and disintegration or sloughed off if the rootlet begins secondary growth. Hyphae and attached vesicles would then appear in the soil as the extramatrical mycelium. A life cycle of this sort has definite survival value for the fungus and would provide a logical mechanism for the conservation of nutrients which otherwise might become unavailable to the green plant. Such a sequence would explain some of the difficulties which have prevented the general acceptance of a life cycle for a vesicular–arbuscular mycorrhiza of this type. Among these difficulties have been the following.

1. The ephemeral nature of the connection between extra- and intramatrical mycelium (18).
2. The absence of visual observations of growth of the extramatrical mycelium in nature.
3. The association of extramatrical mycelium with organic material (decaying rootlet tissue?) in the soil (17).
4. The almost universal presence of vesicles, arbuscules, and hyphae together in the same rootlets.
5. The presence of arbuscules (haustoria) in host cells.
6. The attachment of numerous thin-walled empty hyphae on the extramatrical mycelium. These may be the remains of arbuscules and the hyphae which led to them.

An investigation to prove or disprove this hypothesis is under way.

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